Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Beta-Mannosidase Activity Assay Kit (Fluorometric)

04/20

(Catalog # K2045-100; 100 assays; Store at -20°C)

I. Introduction:

Beta-mannosidase (β-Man; EC 3.2.1.25) is an enzyme that hydrolyzes terminal, non-reducing beta-D-mannose residues in beta-D-mannosides. It is a member of the glycoside hydrolase family 2. There are numerous applications of β-Man including the synthesis of glycosides, production of fermentable sugars, curing mannosidosis, etc. Lysosomal β-Man is essential for catabolizing the Asn-linked glycans of glycoproteins thereby maintaining the cellular homeostasis. β-Man deficiency results in Beta-Mannosidosis, a rare inherited disorder affecting the break-down of mannose-containing disaccharides. These disaccharides gradually accumulate in the lysosomes and cause cells to malfunction. **BioVision's Beta-Mannosidase Activity Assay Kit** provides a facile, rapid way to monitor total β-Man activity in various biological samples. In this kit, β-Man cleaves a synthetic specific substrate and releases a fluorophore, which can be easily quantified (Ex/Em = 360/445 nm). The substrate is specific to β-Mannosidase and can differentiate from α-Mannosidase. The assay is simple, sensitive and can detect as low as 1.0 µU of beta-mannosidase activity.

β-Mannosidase Substrate $\xrightarrow{\text{β-Mannosidase}}$ Cleaved Substrate + Fluorescent Product (Ex/Em = 360/445 nm)

II. Application:

- Measurement of beta-mannosidase activity in various samples

III. Sample Types:

- Biological fluids: Human plasma, etc.
- Cell Lysates: 3T3 cells, etc.

IV. Kit Contents:

Components	K2045-100	Cap Code	Part Number
β-Man Assay Buffer	25 ml	NM	K2045-100-1
β-Man Stop Buffer	25 ml	WM	K2045-100-2
β-Man Substrate	100 µl	Blue	K2045-100-3
4-Methylumbelliferone Standard	35 µl	Yellow	K2045-100-4
β-Man Positive Control	1 vial	Green	K2045-100-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (plate reader)
- 96-well clear plate with flat bottom
- Dounce Tissue Homogenizer (BioVision Cat. # 1998)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **β-Man Assay Buffer and β-Man Stop Buffer:** Store at 4°C or -20°C. Bring to room temperature (RT) before use.
- **β-Man Substrate:** Light sensitive, protect from light. Thaw at RT. Store at -20°C.
- **4-Methylumbelliferone Standard (5 mM):** Light sensitive, protect from light. Thaw at RT. Store at -20°C.
- **β-Man Positive Control:** Reconstitute with 100 µl β-Man Assay Buffer and mix thoroughly. Divide into aliquots & store at -20°C. Keep on ice while in use. Avoid multiple free-thaw cycles. Use within two months.

VII. β-Mannosidase Activity Assay Protocol:

1. **Sample Preparation: For tissue and cells:** Homogenize tissues (10 mg) or pelleted cells (~5 x 10^5) with 100 µl ice-cold β-Man Assay Buffer using Dounce Tissue Homogenizer (BioVision Cat. # 1998) and keep on ice for 10-15 min. Centrifuge samples at 12,000 x *g* and 4°C for 10 min and collect the supernatant. Dilute the supernatant 5-10 fold in β-Man Assay Buffer. Add 2-10 µl of the diluted samples into a 96-well clear plate designated as Sample(s). **For Biological Fluids:** Undiluted fluids can be added directly to the wells. Add 2-10 µl of samples into well(s) of a 96-well clear plate designated as **Samples**.

   For **Reagent Background Control well:** Add 2-10 µl of β-Man Assay Buffer in parallel well(s).

   For **Positive Control well:** Dilute the reconstituted β-Man Positive Control 10 fold with β-Man Assay Buffer prior to the assay. Add 2-6 µl of the diluted β-Man Positive Control into desired wells(s).

   Adjust the volume of **Positive Control**, **Sample(s)**, and **Reagent Background Control** wells to **40 µl/well** with β-Man Assay Buffer.

   Notes:

   a. We suggest running several dilutions of the Samples to ensure the readings are within the Standard Curve range.
   b. Do not re-use the diluted β-Man Positive Control.

2. **Standard Curve Preparation:** Prepare a 100 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µl of 4-MU stock solution to 490 µl β-Man Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl of 100 µM 4-MU Standard into a series of wells to generate 0, 200, 400, 600, 800, 1000 pmol/well of 4-MU Standard respectively. Adjust the volume to **60 µl/well** with β-Man Assay Buffer.

3. **Substrate Hydrolysis:** Prepare sufficient volume of 20-fold dilution of the β-Man Substrate immediately before the assay (i.e. dilute 10 µl of β-Man Substrate with 190 µl of β-Man Assay Buffer), vortex briefly. Add 20 µl of the diluted β-Man Substrate to each well containing the Sample(s), Positive Control and Reagent Background Control. The total volume of each well including Samples, Positive Control and Reagent Background Control should be **60 µl**. **Mix well and incubate at 37°C for 20 min, protected from light**. After incubation, add 200 µl of β-Man Stop Buffer to all the wells including Sample(s), Positive Control, Reagent Background Control, and Standards. Mix well.

   Notes:

   a. Prepare the diluted β-Man Substrate solution immediately before the assay and do not re-use the diluted solution.

   b. Standards can be prepared at the end of the incubation time and measured in end-point mode.

4. **Measurement:** Measure fluorescence intensity of all wells at 37°C in end-point mode at Ex/Em = 360/445 nm.

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the 4-MU Standard Curve. Subtract the Reagent Background Control reading from all Sample readings to get the corrected Sample readings (ΔRFU). Apply the corrected Sample readings (ΔRFU) to 4-MU Standard Curve to obtain the corresponding pmol of product formed **(B, in pmol)** and calculate the activity of β-Mannosidase in the Sample as:

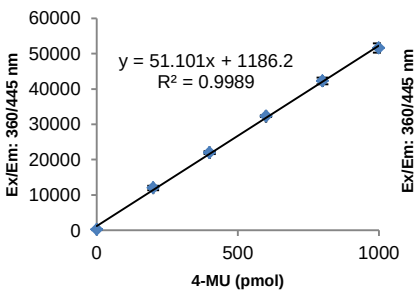
Sample β-Mannosidase Activity = B x 3/ (V x P) x D (pmol/hr/mg ≡ 0.0167 µU/mg)

Where: **B** = 4-MU amount in the Sample well from the Standard Curve (pmol)
3 = Inverse of reaction time (hr)
V = Sample volume added into the reaction well (ml)
P = Initial Sample concentration (mg/ml)
D = Sample dilution factor (D= 1 for undiluted samples)

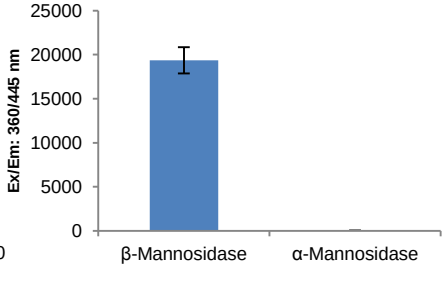
1 pmol/hr = 0.0167 pmol/min = 0.0167 µU

Unit Definition: One unit of beta-mannosidase activity is the amount of enzyme that generates 1.0 µmol of 4-Methylumbelliferone per min, at pH 4.5 at 37°C.

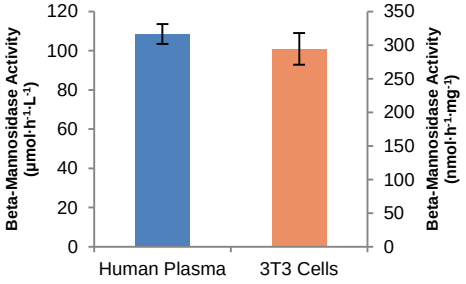
a)



b)



c)



Figures: (a). 4-Methylumbelliferone (4-MU) Standard Curve. (b). Measurement of purified β-Mannosidase (5 ng) and α-Mannosidase (6 ng) activities using BioVision's proprietary substrate. The kit can efficiently distinguish β-Mannosidase activity from α-Mannosidase. (c). β-Mannosidase activity in human plasma (10 µl) and 3T3 cells (2 µg protein). All assays were performed following the kit protocol.

VIII. Related Products:

Alpha-Mannosidase Activity Assay Kit (Fluorometric) (K2041)
Alpha Galactosidase (β-Gal) Activity Assay Kit (Fluorometric) (K407)
Beta Galactosidase (β-Gal) Activity Assay Kit (Fluorometric) (K821)
Glucosylceramidase Activity Assay Kit (Fluorometric) (K2003)
Alkaline Sphingomyelinase Activity Assay Kit (Colorimetric) (K987)
Acid Sphingomyelinase Assay Kit II (Colorimetric) (K192)
β-L-Fucosidase (FUCA1) Assay Kit (Colorimetric) (K224)
β-Glucosidase Activity Colorimetric Assay Kit (K690)
Dounce Tissue Homogenizer (1998)

FOR RESEARCH USE ONLY! Not to be used on humans